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Genetic polymorphism of GH; PIT 1 and PRLR genes in six lines of Sudanese chickens

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Abstract:
In the present study, three genes named: GH; PIT 1 and PRLR were genotyped using PCR-RFLPs technology in six Sudanese chicken lines: Bare neck (1; 2); Large Baladi (1; 2); Frizzle and Betwil. For each line, twenty blood samples were collected from wing vein; DNA was extracted, PCRs were performed using specific primers for the genes understudy, the PCR products were characterized after cutting their products with previously reported restriction enzymes. The results showed the absence of polymorphism for the PRLR gene which shows only one allele or genotype (B; BB) while the other two genes were polymorphic. The GH gene showed two alleles (A; B) with three genotypes (AA; AB; BB), while the PIT 1 gene showed also two alleles (A; B) with only two genotypes (AA; AB), frequencies of alleles and genotypes were varied according to the line. In conclusion the GH and PIT 1 genes can be used as candidate genes for genetic improvement of Sudanese chickens while PRLR gene cannot be due to the lack of polymorphism in it.
Keywords: PIT 1; GH; PRLR; polymorphism, Sudanese, chickens

Introduction:

The Sudanese indigenous chicken breeds have been classified and described by [1] into four main types with some subsidiary lines into: Large Baladi (1; 2); Bare Neck (1; 2); Betwil and Frizzle. Theses chicken are differ much in phenotype and productivity.

Recent advances in molecular biology enabled the scientists to determine the some genes linked with productivity traits in poultry. These genes are called candidate genes [2]. The identification and utilization of potential candidate genes with significant effects on economically important traits have become increasingly important in poultry breeding programs. They could be considered as a marker for quantitative trait loci (QTL) and can be used for genetic improvement through the genotype assisted selection (GAS) [3].

From the candidate genes used in poultry breeding: Pituitary-specific transcription factor (PIT-1); growth hormone (GH) and prolactin receptor (PRLR).

Growth hormone (GH) axis and the transforming growth factor-beta subfamily are the most important groups of genes that are involved in a wide variety of physiological functions such as growth, and reproduction [4, 5, 6]. The chicken growth hormone (cGH) gene is considered as one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism [7]. GH gene expression in pituitary somatotrophs depends on a pituitary-specific transcription factor, Pit-1 (also called GHF-1), which is responsible for tissue-specific expression of genes encoding GH [8].

Pituitary specific transcription factor 1 (pit-1) is a transcription factor involved in binding with the promoters of growth hormone, prolactin and thyroid stimulating hormone-beta genes, thus helps in expression of the genes in chicken [9]. Pit-1 factor participates in controlling immune system of the body through mechanism of growth hormone cascade in birds. The pit-1 factor is also involved in development of anterior pituitary gland, silencing adrenarche [10] and inducing differentiation of hepatic progenitor cells into prolactin-producing cells [11].
The biological actions of PRL are reported to be mediated by a prolactin receptor (PRLR), which shares relatively high degree of structural similarity with growth hormone receptor (GHR) and belongs to the class I cytokine receptor superfamily [12, 13]. Activation of PRLR by PRL binding can initiate the intracellular signaling cascades, including activation of the intracellular JAK2-STAT5 signaling pathway [13, 14]. Recently, it is discovered that the PRLR gene is expressed in the chicken granulose cells [15].

The prolactin receptor (PRLR) has an important role in the PRL signal transduction cascade, which is triggered at the onset of broodiness, and PRL exerts its biological functions by acting through the PRLR [13]. PRLR is also an important regulator gene for cell growth and differentiation. Because

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the PRLR gene is on the chicken Z chromosome [16], the hypothesis of sex-linked inheritance of broodiness may relate to PRLR gene via its role in PRL signal transduction. The identified polymorphism of this gene is mainly viewed in terms of egg production traits.

The aim of the present study is to identify the genetic polymorphism for three genes (PIT-1; GH and PRLR) linked with some productive traits in six Sudanese chicken lines {Bare neck (1; 2); Large Baladi (1; 2); Frizzle and Betwil}, the possible use of these genes in genetic improvement through Genotype assisted selection (GAS). .

Materials and Methods:

Hundred and twenty blood samples were collected from six different Sudanese indigenous chicken lines. The chickens were randomly selected from pure adult unrelated individuals. The samples were collected from different five ecological zones which were Northern Upper Nile Area (Southern Sudan) where (BN1) Bare neck were collected, Butana Area, Gadarif State (Northern Sudan) where (BN2) Bare neck and LB1 Large Baladi were collected, Abu Naama Area, Sinnar State (Northen Sudan) where (LB2) Large Baladi were collected, Lagawa Area, Western Kordofan Area (Northern Sudan) where (FZ) were collected and Nuba Mountains Area where (BT) Betwil were collected. The blood samples were collected on a vacutainer tubes supplied with EDTA (as an anticoagulant). Genomic DNA was isolated and purified using standard Extraction Accuprep® Genome DNA Extraction kit (Bioneer Corporation, South Korea) using the manufacture instructions. DNA concentrations were measured using UV spectrophotometer on 260 nm wavelength, diluted to 50 ng to be ready for amplification.

For Genotyping the growth hormone (PMI region), the protocol of [17] was followed. The forward and the reverse primer sequences were: CTAAAGGACCTGGAAGAAGGG and AACTTGTCGTAGGTGGGTCTG. The PCR program was: initial denaturation at 95°C for 4 min, 35 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and an extension at 72°C for 7 min. The amplification produced a fragment of 1050 bp, this fragment was subject to be cut with Sac1 restriction enzyme according to the manufacture instructions.

Genotyping the intron 5 of the PIT 1 gene was done following the protocol of [18]. The forward and the reverse primer sequences were: GGACCCTCTCTAACAGCTCTC and GGGAAGAATACAGGGAAAGG. The PCR program was: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 62°C for 45 sec, 72°C for 1 min, and an extension at 72°C for 5 min. The amplification produced a fragment of 599 bp, this fragment was subject to be cut with Taq1 restriction enzyme according to the manufacture instructions.

Regarding the genotyping of exon 5 of the PRLR gene, the methodology described by [19] was followed. The sequences of the forward and the reverse primers were: TTGTCTGCTTTGATTCATTTCC and TGCATTTCATTCTTCCCTTTTT. The PCR program was: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 59°C for 1 min, 72°C for 1 min, and an extension at 72°C for 5 min. The amplification produced a fragment of 250 bp, this fragment was subject to be cut with BamH1 restriction enzyme according to the manufacture instructions.

The PCR cocktail was the same for the three genes studied; it was performed in a final volume of 20 µl containing 100 ng of genomic DNA, 0.5 µM of each primer, 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$, 1.0 U *Taq* DNA polymerase and 1× reaction buffer.

To confirm the PCR success, 5ul from the PCR product were subject to run horizontally on 2% agarose gel in TBE buffer, at 110 volt for 25 min., then stained with ethidium bromide. The bands were visualized using UV transilluminator. For identification of the genetic alleles PCR products of the genes were subject to cut with appreciate restriction enzyme depending on the gene itself. The cut was done using the enzyme manufacture instructions.

The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). Gels were visualized under UV light and documented using gel documentation system (BIO-RAD).

Results:

Amplification of prolactin receptor gene (*PRLR*) produced a band size of 250 bp (Fig. 1), after cutting with BamH1 restriction enzyme, two bands size of 195 and 55 (representing allele B) were observed in all the line samples studied (Fig. 2), meaning the presence of only one allele (B) and one homozygous genotype (BB), subsequently the

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absence of polymorphism in this gene in all the lines studied.

From another side the two other genes studied showed polymorphism in all the lines studied. Amplification of growth hormone gene (GH) produced a band of 1050 bp (Fig. 3), after cutting with Sac 1 restriction enzyme, two bands (representing allele B) were observed in some line samples studied (Fig. 4), meaning the presence of two alleles (A, B) two homozygous genotypes (AA; BB), and one heterozygous genotype (AB). Results of allele frequencies as well as genotype frequencies are presented in table 1.

The amplification of the Pituitary specific transcription factor 1 gene (PIT 1) produced a band of 599 bp (Fig. 5), after cutting with Taq 1 restriction enzyme, two bands (representing allele B) were observed in some line samples studied (Fig. 6), meaning the presence of two alleles (A, B). Only genotypes were observed, the first was the homozygous genotype (AA) while the second genotype was the heterozygous genotype (AB), the homozygous genotype (BB) did not observed in any line. Results of allele frequencies as well as genotype frequencies are presented in table 2.

Allele sharing results (in Table 2) showed that the alleles A, B were shared in all the lines studied for both GH and PIT1 genes, while the only found and shared allele for the PRLR gene was the B allele.

Analysis of each studied gene alleles (No, observed number of alleles; Ne, effective number of alleles), heterozygosity (Ho, observed; He, expected) and polymorphism information content (PIC) for each line under study is presented at table 3.

Results of average gene diversity over loci in the six chicken lines are presented at table 4.

Regarding the inbreeding estimates for the three genes studied in the six lines (FIS), it was noticed all the values were below zero or negative meaning the absence on inbreeding with the each line (table 5).

Similar results were observed for the inbreeding values between the six line studied (FST). These results are presented at table 6.

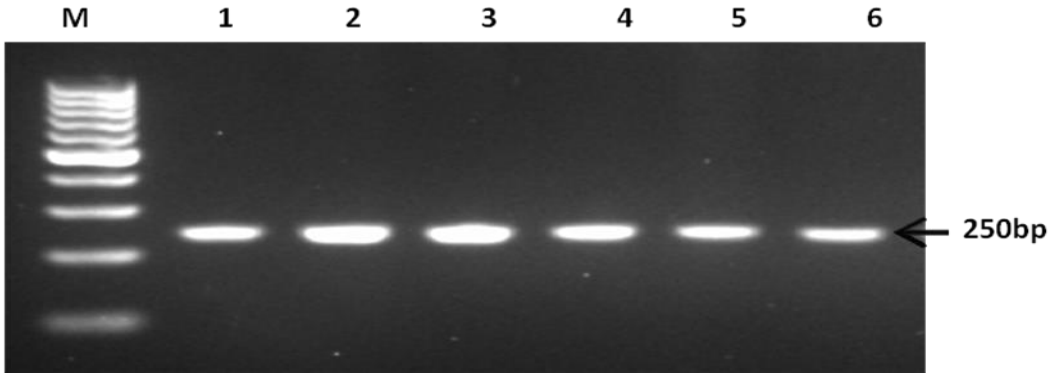

Figure 1: PCR products representing amplification of *PRLR* gene in Sudanese chicken. Lane M: 100 bp ladder marker.

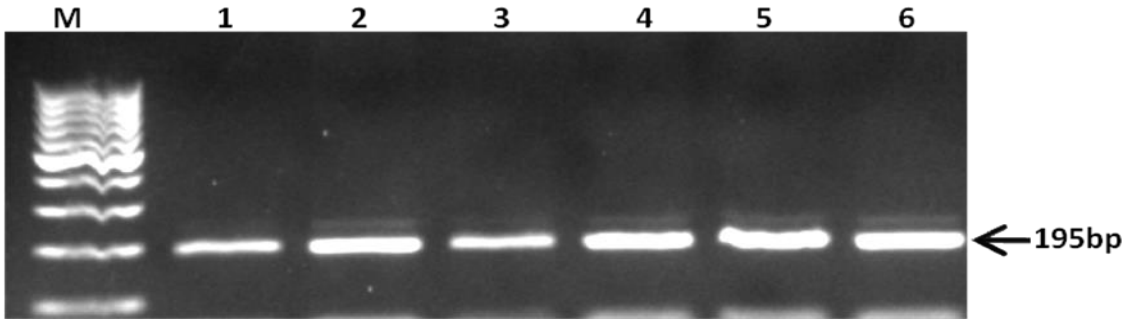

Figure 2: PCR products of *PRLR* gene after cutting with BamH1 restriction enzyme. Lane M: 100 bp ladder marker.

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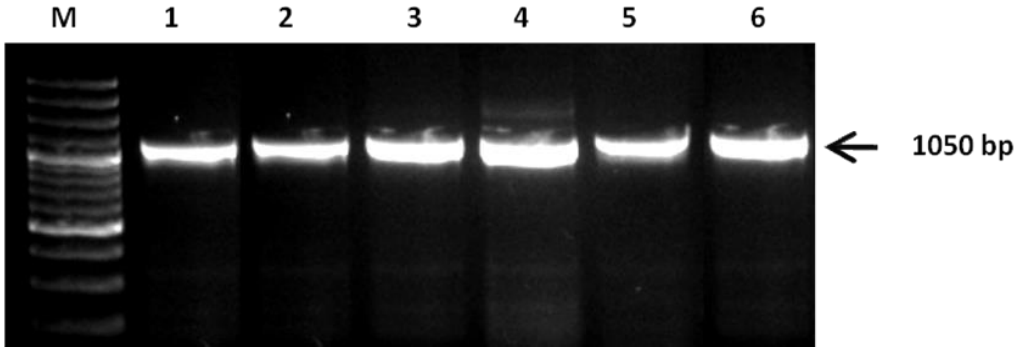

Figure 3: PCR products representing amplification of *GH* gene in Sudanese chicken. Lane M: 100 bp plus ladder marker.

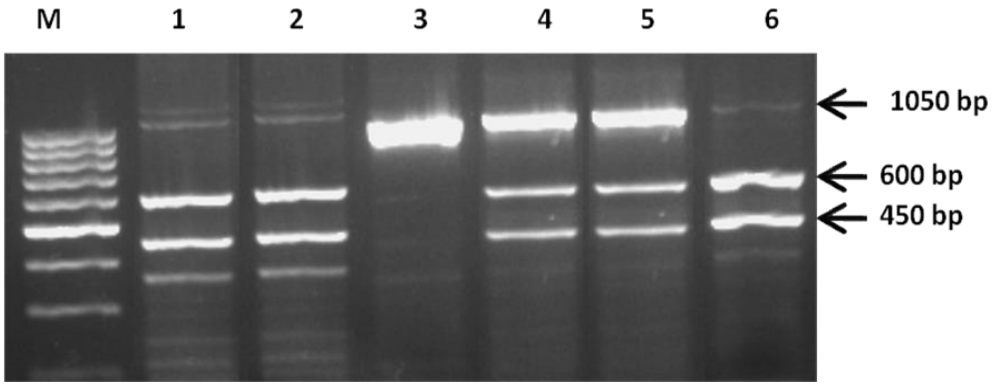

Figure 4: PCR-RFLP pattern for *GH* gene with Sac 1 digestion. Lane M: 100 bp ladder marker. Lane 3: homozygous genotype (AA) with one fragment at 1050 bp, Lanes 1, 2& 6: homozygous genotype (BB) with two digested fragments at 600 and 450-bp, Lanes 4&5: heterozygous genotype (AB) with three digested fragments at 1050, 600 and 450 bp.

Table 1. Frequencies of alleles and genotypes and of GH gene in Sudanese chicken lines.

Strain	Line	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
Bare neck	BN1	0.500	0.500	0.313	0.375	0.313
	BN2	0.375	0.625	0.090	0.580	0.330
Large Baladi	LB1	0.500	0.500	0.330	0.330	0.330
	LB2	0.500	0.500	0.270	0.460	0.270
Frizzle	FZ	0.563	0.438	0.125	0.875	0.000

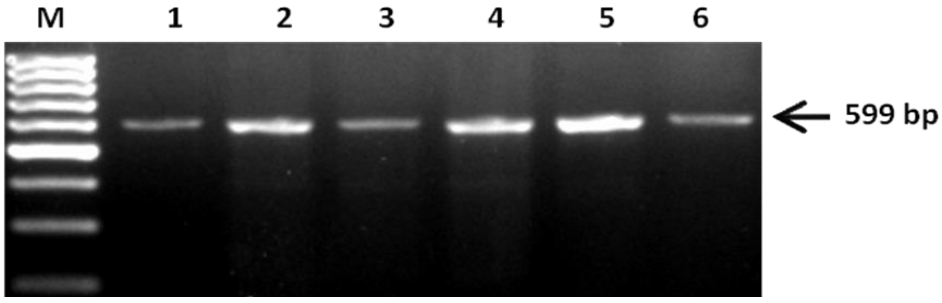

Figure 5: PCR products representing amplification of *PIT 1* gene in Sudanese chicken. Lane M: 100 bp ladder marker.

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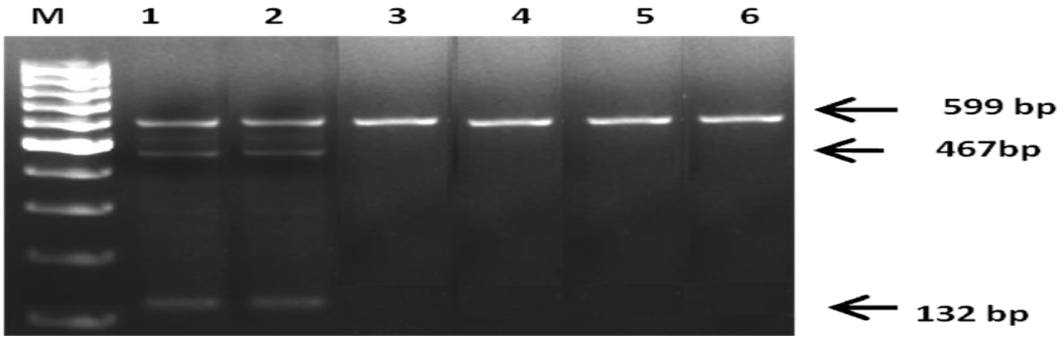

Figure 6: PCR-RFLP pattern for *PIT 1* gene with *Taq* 1 digestion. Lane M: 100 bp ladder marker. Lane 3, 4, 5 &6: homozygous genotype (AA) with one fragment at 599-bp, Lanes 1&2: heterozygous genotype (AB) with three digested fragments at 599,467 and 132-bp.

Table 2. Frequencies of alleles and genotypes of *PIT 1* gene in Sudanese chicken lines.

Strain	Line	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
Bare neck	**BN1**	0.944	0.056	0.888	0.111	0.000
	BN2	0.962	0.039	0.923	0.077	0.000
Large Baladi	**LB1**	0.944	0.056	0.889	0.111	0.000
	LB2	0.938	0.062	0.875	0.125	0.000
Frizzle	**FZ**	0.900	0.100	0.800	0.200	0.000

Table 3. Allele, heterozygosity analysis and polymorphism information content for the studied genes in Sudanese chicken lines.

Breed /Gene			PRLR	GH	PIT1
BN1	Alleles	No	1	2	2
		Ne	1.000	2.000	1.117
	Het.	Ho	1.000	0.375	0.111
		He	0.000	0.516	0.108
	PIC		0.000	0.375	0.100
BN2	Alleles	No	1	2	2
		Ne	1.000	1.882	1.080
	Het.	Ho	1.000	0.583	0.0769
		He	0.000	0.489	0.077
	PIC		0.000	0.359	7.022
LB1	Alleles	No	1	2	2
		Ne	1.000	2.000	1.117
	Het.	Ho	1.000	0.333	0.111
		He	0.000	0.517	0.108
	PIC		0.000	0.375	0.100
LB2	Alleles	No	1	2	2
		Ne	1.000	2.000	1.133
	Het.	Ho	1.000	0.467	0.125
		He	0.000	0.517	0.121
	PIC		0.000	0.375	0.110
BT	Alleles	No	1	2	2
		Ne	1.000	1.969	1.220
	Het.	Ho	1.000	0.875	0.200
		He	0.000	0.508	0.186
	PIC		0.000	0.370	0.164
FZ	Alleles	No	1	2	2
		Ne	1.000	1.960	1.095
	Het.	Ho	1.000	0.571	0.091
		He	0.000	0.508	0.091
	PIC		0.000	0.370	8.226

No: number of observed alleles, Ne: number of expected alleles, Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphism information content

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Table (4): Average gene diversity over loci per studied lines.

Line	Gene			Mean estimate
	PRLR	GH	PIT1	
BN1	0.000	0.516	0.107	0.208
BN2	0.000	0.489	0.077	0.189
LB1	0.000	0.517	0.108	0.209
LB2	0.000	0.517	0.121	0.213
BT	0.000	0.508	0.186	0.231
FZ	0.000	0.508	0.091	0.200
Total the breeds	0.000	0.502	0.114	0.205

Table (5): Inbreeding estimates (FIS = *f*) within populations under study.

Gene	Line					
	BN1	BN2	LB1	LB2	BT	FZ
PRLR	0.000	0.000	0.000	0.000	0.000	0.000
GH	0.250	-0.2500	0.333	0.067	-0.778	-0.167
PIT1	-0.059	-0.0588	-0.059	-0.067	-0.111	-0.048

Table (6): Inbreeding rates between populations under study (FST).

Breed	BN1	BN2	LB1	LB2	BT	FZ
BN1	******	0.0120-	-0.0416	0.0381-	0.0163-	-0.0288
BN2	-0.0120	******	0.0144-	-0.0094	0.0398	0.0296-
LB1	-0.0416	-0.0144	******	-0.0403	-0.0177	-0.0310
LB2	-0.0381	-0.0094	-0.0403	******	-0.0157	-0.0268
BT	-0.0163	0.0398	-0.0177	-0.0157	******	0.0100
FZ	-0.0288	-0.0296	-0.0310	-0.0268	0.0100	******

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table (7): FST (Fisher statistics) estimate (below diagonal) and effective migration rate *(Nm)* (above diagonal) (FST=1/4*N* m+1) (Wright 1951).

Line	BN1	BN2	LB1	LB2	BT	FZ
BN1	****	0.994	1.000	1.000	0.998	0.999
BN2	0.007	****	0.994	0.993	0.984	0.999
LB1	0.000	0.007	****	1.000	0.998	0.998
LB2	0.000	0.007	0.000	****	0.998	0.998
BT	0.003	0.016	0.002	0.002	****	0.991
FZ	0.002	0.001	0.002	0.002	0.009	****

Discussion:

Genetic improvement of farm animals, including poultry, is generally aimed at maximizing economic performance and production traits, especially in poultry breeders and table egg producing hens. The candidate gene approach is a very powerful method to investigate associations of gene polymorphisms with economically important traits in farm animals [2]. Many studies have examined growth, skeletal and immune function traits using the candidate gene approach in chickens [20, 21, 22]. Genetic diversity is important in breeding programs for the genetic optimization of the acquisition of certain properties that can be achieved when there is enough opportunity for the selection of genes or the desired properties. In addition, genetic diversity

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plays an important role in the survival of the population. The loss of genetic diversity can reduce the chance of survival of the population. Studies conducted over the last few years provide evidence that PRLR gene, Growth hormone gene (GH) and Chicken PIT1 gene took part, directly and indirectly, in shaping many production traits in poultry, resulting in these genes being considered as candidate markers of these traits.

In the present study, genotyping of *PRLR* gene in the six Sudanese chicken lines did not show any polymorphism and shows only one allele B and subsequently genotype BB. Heterozygous genotype AB was absent also, this absence of heterozygous individuals is expected due to the localization of gene on Z chromosome and subsequently it is a sex linked gene [23].

Similar result was obtained by [24] after typing the *PRLR* gene in Ukrainian Poltava clay chicken which shows only one allele. In the previous studies, authors have suggested that the *PRLR* gene was attractive as a candidate gene for broodiness, egg production and age of first egg in chicken [25, 3, 26]. The chicken *PRLR* gene have mapped as a candidate gene for the control of broodiness [16], because a major gene involved in susceptibility to broodiness is thought to reside on the chicken Z chromosome [27] and manifest as a sex-linked trait. It is found that individuals with AA genotype produced more eggs higher than BB genotype during recorded laying time [19]. Therefore, it was expected that the Sudanese chicken line would produce less egg count as the frequency of allele B was 1.0 while the allele A was absent completely. These results were in disagreement with the study of [19] who found that the frequency of A allele was higher (0.72) than B allele (0.28) at PRLR5 marker site in the Mazandaran Iranian chickens. Prolactin receptor gene is mediating the work of prolactin and growth hormone receptor genes [12, 13]. Presence of one allele for the PRLR gene in the chicken lines understudy will affect productive and reproductive traits of these chickens.

The chicken growth hormone (cGH) gene is considered as one of the most important candidate genes that can influence chicken performance traits because of its crucial function in growth and metabolism [7]. The cGH gene contains 4 exons and 5 introns with an overall length of 4.1 kb and 5.2 kb in the chicken and duck respectively [28]. Polymorphisms in the cGH gene were widely studied by restriction fragment length polymorphisms or DNA sequencing [29]. In the present study polymorphisms of the GH gene were detected with two alleles (A, B) in all the lines genotyped. This is similar to pervious study of [30], who found that chicken growth hormone genes have polymorphisms which can be clarified by digestion with Msp I and Sac I, and suggests that they may have significant association with chicken abdominal fat content. In another studies [29, 31, 32, 33, 34] found an association between the polymorphism of this gene and carcasses composition, growth and fatness. From another side, association between growth hormone alleles and productive traits has been reported in previous study of [35] who found that *GH* genotype was significantly associated with age at first egg as well as the hen-day rate of egg production. Also [36, 17, 37] reported that polymorphism of gene affects egg laying hens; egg production and rate of laying eggs. In a report by [24] it is found that allele A of GH gene is linked with laying more eggs than allele B in the Ukrainian Poltava clay chicken. Moreover [38] found an association between allele C of chicken growth gene hormone (introns 4) with an increase in egg and meat production in Rhode Island chickens. In contrast [39] did not find an association between the alleles of the GH gene (G1705A SNP) and productive traits in broiler Iraqi chickens. In the present study we found almost equal distribution of the two alleles (A, B), this is against what reported earlier by [40] who found that the allele A was the most frequent and ranged from 0.99 to 0.79, and they attribute this observation to the long term selection strategy sued in the population they study.

Due to its crucial regulatory function and a variety of bioactivities, *PIT1* has been regarded as a key candidate gene for production performance. There are indications that variations of *PIT1* gene are related to growth, carcass and fatty traits in pig [41, 42], growth and carcass traits in cattle [43, 44]. According to the chicken genome sequences, this gene is localized on chromosome 1 [45].

In the present study, the *PIT 1* gene showed polymorphism in the six lines studied. Our results of finding the polymorphism of this gene are similar to the earlier reports of this gene polymorphism [46, 18, 47].

Our results showed the presence of two genotypes; AA and AB with the absence of BB genotype in all tested lines for this gene. This is may be due to the predominant of allele A over allele B as reported earlier by [46]. The predominance of allele A over allele B was very clear at our lines studied since allele A frequency usually exceeds 0.9 in the chicken line studied. In precise *PIT 1* seemed to have higher

effects on chicken early growth, as they were associated with average daily gain at 0–4 and 4–8 weeks, body weight at 21, 28, 35 and 42 days, and shank diameters at 63 days, respectively [44]. Polymorphisms in *PIT 1* gene were significantly ($P<0.1$) associated with body growth and body composition traits as reported by [18]. This study suggests that *PIT 1* gene could be a candidate locus or linked to major gene(s) that affects growth and body composition traits in the chicken and is therefore, a potential marker for molecular MAS programs in commercial broiler line.

In conclusion, due to the presence of polymorphism in the Pit 1 and GH genes, their association with egg production and growth traits, they can be use as candidate genes in poultry breeding programs in Sudan while PRLR cannot be due to the absence of polymorphism in it.

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